Electrocomp™ GeneHogs® *E. coli*
One Shot® Electrocomp™ GeneHogs® *E. coli*

Catalog nos. C8080-10, C8080-03, C800-05

Version E
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25-0387

www.invitrogen.com

Overview

Introduction

The information in this manual covers the following kits:

Kit	Reactions	Catalog no.
Electrocomp™ GeneHogs® *E. coli*	25	C800-05
One Shot® Electrocomp™ GeneHogs® *E. coli*	10	C8080-10
	20	C8080-03

Contents

Each kit contains the following:

Kit	Number of Tubes	Volume per Tube
Electrocomp™ GeneHogs® *E. coli*	5	100 µl
One Shot® Electrocomp™ GeneHogs® *E. coli*	11 or 21	50 µl

In addition, the kits contain the following reagents:

Reagent	Electrocomp™ GeneHogs® *E. coli*	One Shot® Electrocomp™ GeneHogs® *E. coli*
Control DNA	5.65×10^{-2} fmoles/µl (6.75 ng/µl) BAC DNA (181 kb) in TE Buffer, pH 8 10 µl	5.65×10^{-2} fmoles/µl (6.75 ng/µl) BAC DNA (181 kb) in TE Buffer, pH 8 10 µl
SOC	2 x 6 ml	6 ml

Genotype

F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G *fhu*A::IS2 (confers phage T1 resistance)

Phage Resistance

GeneHogs® have been tested for resistance to the following phages. (ϕ = phi)

Phage	Resistance
T1, P1, Alpha 3 (ϕX174), Ox6, fd, f1, ϕR	Resistant
ϕv-1, T7, T4, T2	Sensitive

General Handling

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Transformation should be started immediately following the thawing of the cells on ice. Mix by swirling the tube gently, not by pipetting or vortexing.

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Preparation of Ligation Reactions

The salts present in ligation buffers can cause:

- Arcing during electroporation
- Lowered transformation efficiencies when compared to purified plasmid DNA

Ethanol precipitation, washing, and resuspension in salt-free buffer effectively remove salt from ligation reactions containing small inserts. However, performing these procedures on ligation reactions containing large insert DNA is not recommended because these procedures shear DNA.

We recommend performing membrane dialysis of the ligation reaction to remove the salt. Use the procedure below to dialyze your ligation reaction.

1. Float a 25 mm membrane filter with a 0.025 micron pore size (Catalog no. VSWP 025 00, Millipore) on ~30 ml of sterile 0.5X TE Buffer, pH 8 in a 10 cm petri dish.
2. Carefully pipet the ligation mixture onto the surface of the membrane using a sterile, wide-bore pipet tip.
3. Cover the petri dish and dialyze for 2 hours.
4. Carefully transfer the dialyzed ligation reaction from the membrane surface to a sterile microcentrifuge tube using a sterile, wide-bore pipet tip. Proceed to electroporation.

For transformation of large plasmids, we recommend electroporation for high transformation efficiency and less bias toward large plasmids.

Transformation of Electrocomp™ GeneHogs® *E. coli*

Materials Supplied by the User

Be sure to have the following equipment and reagents on hand before performing transformation.

- Electroporator and cuvettes
- 1.5 ml sterile microcentrifuge tubes
- Sterile Pasteur or transfer pipets
- 37°C shaking and non-shaking incubator
- 100 mm diameter LB agar plates with appropriate antibiotic
- Additional SOC medium (see page 5)
- Ice bucket with ice

Preparation

For each transformation, you will need 20 µl of competent cells and at least two selective plates. Note that each tube of Electrocomp™ GeneHogs® will yield enough cells for 5 transformations.

1. Bring SOC medium to room temperature.
2. Warm selective plates at 37°C for 30 minutes.
3. Place cuvettes and microcentrifuge tubes on ice.
4. Thaw the appropriate number of Electrocomp™ GeneHogs® *E. coli* on ice.

Procedure

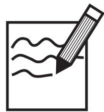
1. Set up your electroporator for bacterial transformation. Follow the manufacturer's instructions.
2. Add 1 µl of each ligation reaction to 20 µl of cells. Stir gently with pipette tip. DO NOT MIX BY PIPETTING UP AND DOWN. **Note**: Unused cells may be refrozen and reused. The transformation efficiency will be lower when using refrozen cells.
3. Transfer the cells to the chilled electroporation cuvette on ice.
4. Dry off the outside of the electroporation cuvette and place the cuvette in the electroporator.
5. Electroporate using the manufacturer's recommended protocol.
6. Remove cuvette from the chamber, quickly add 450 µl of SOC, and mix gently.
7. Transfer the solution to a 1.5 ml microcentrifuge tube and shake at 300 rpm for at least 45 minutes at 37°C to allow expression of the antibiotic resistance gene.
8. Dilute the transformation reaction 100-fold with SOC and spread 20 to 150 µl from each transformation on a pre-warmed LB plate containing the appropriate antibiotic. We recommend that you plate two different volumes to ensure well-spaced colonies for counting and picking. The remaining transformation mix may be stored at +4°C and plated out the next day.
9. Incubate the plates overnight at 37°C.
10. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Transformation of One Shot® Electrocomp™ GeneHogs® *E. coli*

Materials Supplied by the User

Be sure to have the following equipment and reagents on hand before performing transformation.

- 37°C shaking and non-shaking incubator
- 10 cm diameter LB agar plates with appropriate antibiotic
- Additional SOC medium (see page 5)
- Ice bucket with ice
- Electroporator and cuvettes
- Sterile Pasteur or transfer pipets
- 15 ml snap-cap tubes (one for each transformation)



Note

One Shot® Electrocomp™ cells are supplied in 50 µl single-use aliquots. Please refer to the user manual included with your electroporator for cuvette size and reaction volume. You may dispose of any unused cells.

Preparation

For each transformation, you will need one vial of competent cells and at least two selective plates.

1. Bring SOC medium to room temperature.
2. Warm selective plates at 37°C for 30 minutes.
3. Place cuvettes on ice.
4. Thaw **on ice** 1 vial of One Shot® Electrocomp™ cells for each transformation.

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Transformation of One Shot® Electrocomp™ GeneHogs® *E. coli*, Continued

Procedure

1. Set up your electroporator for bacterial transformation. Follow the manufacturer's instructions.
2. Add 1-2 µl of each ligation reaction to the volume of cells recommended by the manufacturer (may be less than 50 µl). Stir gently with pipette tip. **Do not mix by pipetting up and down**.
3. Transfer the cells to the chilled electroporation cuvette on ice.
4. Dry off the outside of the electroporation cuvette and place the cuvette in the electroporator.
5. Electroporate as per the manufacturer's recommended protocol.
6. Quickly add 250 µl room temperature SOC medium and mix gently.
7. Transfer the solution to a 15 ml snap-cap tube (i.e. Falcon) and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance gene.
8. Dilute the transformation reaction 100-fold and spread 20 to 150 µl from each transformation on a pre-warmed LB plate containing the appropriate antibiotic. We recommend that you plate two different dilutions or volumes to ensure well-space colonies for counting and picking. The remaining transformation mix may be stored at +4°C and plated out the next day, if desired.
9. Incubate the plates overnight at 37°C.
10. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Transformation Control

Transformation Control

A BAC plasmid (181 kb) is supplied as a control plasmid. The concentration is 5.65 x 10^{-2} fmoles/µl in TE Buffer, pH 8. Transform 1 µl using the appropriate procedure for the kit you are using and dilute the transformation reaction 100-fold with SOC. Plate 50 µl on LB agar plates containing 12.5 µg/ml chloramphenicol.

Calculation

Calculate the transformation efficiency as transformants per fmole of plasmid DNA. Use the formula below to calculate transformation efficiency:

$$\frac{\text{\# of colonies}}{\text{0.0565 fmoles transformed DNA}} \times \frac{\text{Total transformation volume}}{\text{X µl plated}} \times 100 = \frac{\text{\# transformants}}{\text{fmoles plasmid DNA}}$$

Expected efficiency should be ~1 x 10^6 cfu/fmole plasmid DNA. This is equivalent to ~1 x 10^7 cfu/µg DNA.

SOC Medium

Composition- for 1 liter

2% Tryptone
0.5% Yeast Extract
0.05% NaCl
2.5 mM KCl
10 mM $MgCl_2$
20 mM glucose

1. Dissolve 20 g Tryptone, 5 g Yeast Extract, and 0.5 g NaCl in 950 ml water.
2. Make a 250 mM KCl solution by dissolving 1.86 g of KCl in 100 ml of deionized water. Add 10 ml of this stock KCl solution to the above solution.
3. Adjust pH to 7.0 with 5 M NaOH, and then bring the volume to 980 ml with deionized water.
4. Prepare 1 M $MgCl_2$ by dissolving 20.33 g in 100 ml deionized water.
5. Autoclave both solutions at 15 lbs./sq. in. for 20 minutes.
6. Make a 2 M solution of glucose by weighing out 36 g glucose and dissolving in a final volume of 100 ml deionized water. Filter-sterilize this solution.
7. Let the autoclaved solutions cool to about 55°C, then add 10 ml of the filter-sterilized 2 M glucose solution and 10 ml 1 M $MgCl_2$. Add antibiotic if needed. Store at room temperature or +4°C.

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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